BACTERIAL SOURCE TRACKING (BST) ON TRIBUTARIES OF TRINITY AND GALVESTON BAYS

Texas Commission of Environmental Quality (TCEQ) Project 582-18-80240

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List of Acronyms and Abbreviations

BST	Bacterial Source Tracking
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
ERIC	Enterobacterial Repetitive Intergenic Consensus Sequence
mTEC	Modified Membrane Thermotolerant *E. coli* Medium
MUG	Methylumbelliferyl-b-D-glucuronide
NA	Nutrient Agar
PCR	Polymerase Chain Reaction
RP	RiboPrinting
SAML	Soil and Aquatic Microbiology Laboratory
TCEQ	Texas Commission on Environmental Quality
TMDL	Total Maximum Daily Load
TSSWCB	Texas State Soil and Water Conservation Board
TWRI	Texas Water Resources Institute
UTSPH EP	University of Texas School of Public Health, El Paso
WPP	Watershed Protection Plan

Executive Summary

To better characterize sources of bacteria that are entering the Trinity and Galveston Bays, a BST study was employed to help decision makers determine the most appropriate management measures needed to reduce bacteria in the waterbodies. Monthly sampling was conducted by the Texas Water Resources Institute (TWRI) at one site on Buffalo, Double, Cedar and Dickinson Bayous and Clear Creek for one year (60 total water samples). Samples were delivered to the Soil and Aquatic Microbiology Laboratory (SAML) for processing in preparation for BST. TWRI worked with local experts to determine key sources of bacteria within the watersheds. Using this input, TWRI determined approximately how many samples were needed from various sources of bacteria. A total of 91 known source samples were collected and delivered to SAML. SAML used these samples to supplement the Texas *E. coli* BST Library with isolates DNA fingerprinted using the Enterobacterial Repetitive Intergenic Consensus Polymerase (ERIC-PCR) and RiboPrinting (RP) combination method. SAML also conducted library-dependent BST and analyzed 241 *E. coli* isolates from the 60 water samples (4 isolates per sample) collected.

Major goals of the project were:

1. Expand the Texas *Escherichia coli* (*E. coli*) BST Library through known source sample collection in the Trinity River and Galveston Bay watersheds.
2. Conduct BST analysis for water samples from the Trinity River and Galveston Bay watersheds.

Major findings from the project include:

- The Texas *E. coli* BST Library was expanded and refined, with the current version containing 1,886 isolates from 1,645 known source fecal samples retrieved from 4,301 individual known source samples in over 20 watersheds. Of the 77 known source isolates fingerprinted from the Trinity River and Galveston Bay watersheds, an additional 33 isolates from 31 fecal samples were added to the BST Library.
- BST analysis in the Trinity River and Galveston Bay indicate that wildlife (non-avian and avian) were the leading contributors of *E. coli* in the five selected sample sites with variable levels of human contributions across the sites.

Introduction

Bacterial impairments make up the majority of impairments to water bodies across the state of Texas. The *2018 Texas Integrated Report and 303(d) List* indicates that of the 1,071 water bodies assessed, 574 are impaired. Of those 574 impairments, 237 are impaired for bacteria or roughly 39% of total impairments. Identifying and assessing the sources of bacteria is critical to targeting best management practices, developing bacterial total maximum daily loads (TMDLs) or watershed protection plans (WPPs), and assessing risks from contact recreation. For this project, a complete BST analysis was conducted in five watersheds in the Trinity and Galveston Bays to better characterize the sources of bacteria entering those water bodies and help determine the best solutions to addressing their bacteria impairments. This analysis was also intended to support the implementation efforts of the following WPPs and TMDLs:

- Double Bayou WPP;
- Cedar Bayou WPP;
- The Bacteria Implementation Group (BIG) Plan; and
- Dickinson Bayou WPP

Project Significance and Background

Buffalo Bayou, Cedar Bayou, Clear Creek, Dickinson Bayou and Double Bayou, are all located around either the Upper Galveston Bay or Trinity Bay. Each of these water bodies drain directly into the bays and are listed as impaired waterbodies in the *2018 Texas Integrated Report*. The vast majority of the land serviced by the watersheds being examined can be classified as undeveloped, agricultural, or developed land.

To assess and identify different sources contributing to bacterial loadings in these waterbodies, SAML conducted a BST study using samples from five sites across the watersheds. The numerous BST projects in Texas have largely used library-dependent based methods based on DNA fingerprinting of *E. coli*. Based on a multi-year study initiated in 2002 (Casarez et al., 2007), the Texas Bacteria TMDL Task Force selected the two-method approach using ERIC-PCR and RP, as the most accurate and cost-effective method for library-dependent BST (Jones et al., 2009). Another advantage of library-dependent BST over library-independent BST is the ability to detect a wider assortment of sources. There are numerous library-independent makers, targeting *Bacteriodales* and other organisms available, but these markers can only detect a limited range of sources (e.g., ruminant, human, hog, horse, dog, chicken, and cow) and cannot directly detect the impact of all wildlife. Ideally for watershed characterization, a BST method should be able to detect all host sources present and of concern, especially since numerous studies have indicated wildlife is often a major contributor of *E. coli* to watersheds.

The Texas *E. coli* BST Library is dynamic, with new isolates being added with each successive BST project. This has resulted in 3,764 ERIC-RP composite DNA fingerprints from 3,062 samples from over 50 source subclasses of wildlife, domestic animals, and humans, representing a collection effort of over 4,300 samples from over 140 subclasses, with more than 11,000 known source *E. coli* isolates archived.

For more than a decade, the Texas BST Program has successfully identified sources of *E. coli* in watersheds across Texas. Comprehensive BST has been completed by the University of Texas School of Public Health, El Paso (UTSPH EP) and SAML for the following watersheds: (1) Lake Waco and Belton Lake, (2) San Antonio area, (3) Lake Granbury, (4) Buck Creek, (5) Leon and Lampasas Rivers, (6) Little Brazos River tributaries, (7) Big Cypress Creek, (8) Leona River, (9) Attoyac Bayou, (10) Arroyo Colorado, (11) Navasota River, (12) Big Elm Creek, (13) Plum Creek and the (14) the Trinity River in Tarrant Regional Water District's service area. A Texas *E. coli* BST Library has been developed based on known source isolates from these and other (i.e. Upper Trinity River and Upper Oyster Creek) watersheds.

Sample Collection and Processing

Water samples were collected by TWRI beginning in April 2018 thru April 2019. A total of five sampling locations were allocated across the watershed (Figure 1). Monthly monitoring sites included the 5 stream samples. Stream samples included Dickinson Bayou, Cedar Bayou, Buffalo Bayou, Double Bayou and Clear Creek. Sampling over 12 months from five sites and obtaining four isolates from each sample yielded a minimum of 240 isolates to be analyzed using ERIC-RP.

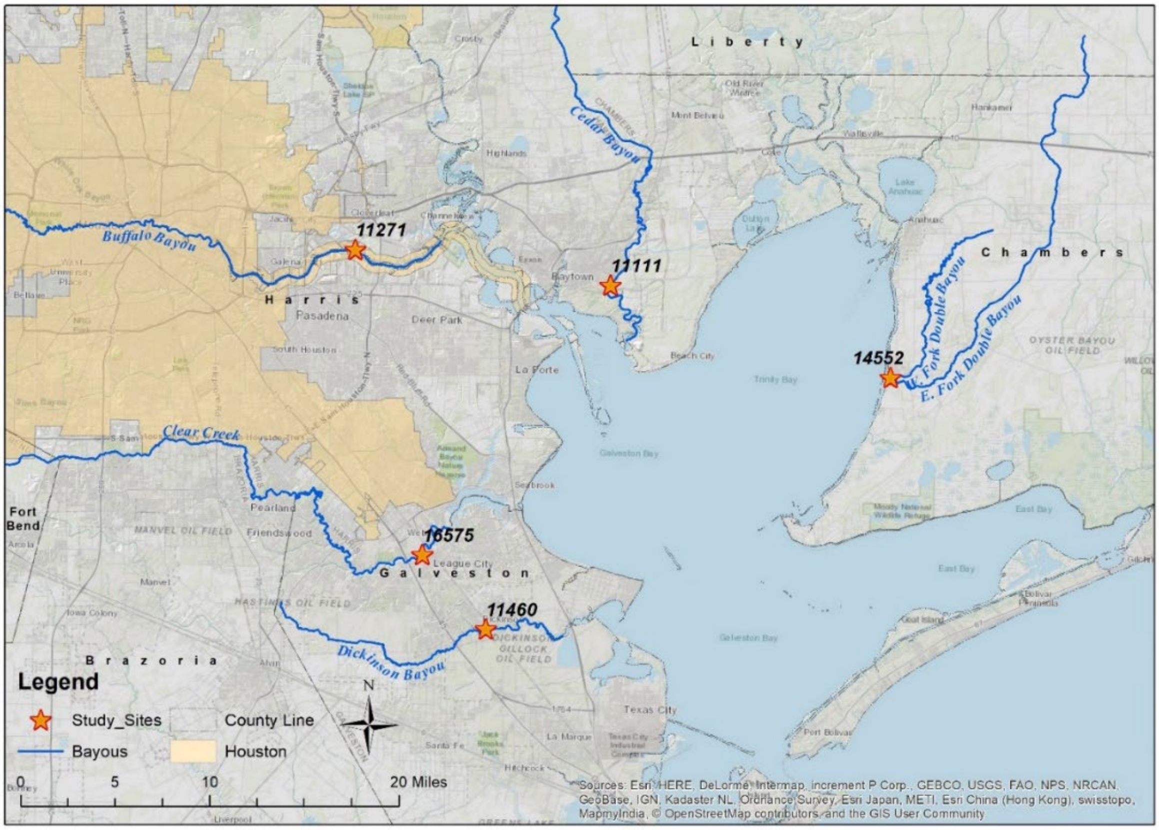

Figure 1. Monitoring sites on the Galveston and Trinity Bay Tributaries.

TWRI collected and SAML processed the water samples for downstream BST analysis within 8 hours of sample collection using UTSPH EP standard operating procedures. For *E. coli* isolations, water samples were processed using the United States Environmental Protection Agency's (USEPA) Method 1603 and modified membrane thermotolerant *E. coli* (mTEC) medium (USEPA 2005). *E.*

coli colonies were then picked from the modified mTEC medium and streaked onto nutrient agar (NA) with Methylumbelliferyl-b-D-glucuronide (MUG) NA in order to confirm culture purity. Cultures of selected isolates were archived at -80°C for subsequent BST analyses.

Known-source fecal samples were also collected by TWRI and transported to SAML for processing. *E. coli* were isolated from the fecal samples and processed and archived using USEPA Method 1603 and UTSPH EP s, as described above for the water samples. In general, no more than three isolates were fingerprinted per fecal sample using ERIC-RP and compared using densitometric curve-based Pearson-product similarity coefficients. Isolates deemed source-specific through self-validation (described below) were added to the Texas *E. coli* BST Library.

DNA Fingerprinting of *E. coli* Isolates

Both ERIC-PCR and RP were performed as previously described by Casarez et al. (2007). The *E. coli* isolates were first DNA fingerprinted using ERIC-PCR (Versalovic et al. 1994). Following ERIC-PCR analysis, *E. coli* isolates were riboprinted using the automated Hygiena RiboPrinter® system and the restriction enzyme *Hin*dIII. Analysis of composite ERIC-RP DNA fingerprints was performed using Applied Maths BioNumerics software (Casarez et al. 2007).

Known source fecal samples were collected as a portion of the BST efforts to add Galveston Bay watershed specific isolates into the Texas *E. coli* BST library. Of the 91 total known source fecal samples collected and processed from the watershed, *E. coli* were successfully isolated from 76 individual samples. All 76 of these isolates (one isolate per known source sample) were screened using ERIC-RP and included in the local watershed library. Jackknife analysis of the ERIC-RP was used to identify isolates that correctly classified using a 7-way split of source classes (i.e., human, pets, cattle, other non-avian livestock, avian livestock, avian wildlife, and non-avian wildlife). Isolates with unique fingerprints (left unidentified using an 80% similarity cutoff) were also included to create the local self-validated library. In total, 46 isolates were self-validated in the local library. Those self-validated local isolates were then combined with similarly screened isolates from all the previous studies. Serial jackknife analysis was performed using a 3-way split of source classes and an 80% similarity cutoff, removing incorrect matches, and then repeating the analysis until there were zero incorrect matches in the library after serial jackknife analysis was complete. After four iterations of cross-watershed validation, the resulting Texas *E. coli* BST Library (ver. 1-20) contained 1,886 isolates from 1,645 samples, resulting in a 100% ARCC with a 3-way split of source classes and a 91% ARCC using the 7-way split of source classes. A total of 19% of the isolates were identified as singletons (unique fingerprints left unidentified using an 80% similarity cutoff) and were kept in the library in order to reflect the diversity of patterns potentially seen in unknown water samples (Table 1).

After cross-watershed validation, 33 isolates (43% of the local library samples) were included in the Texas *E. coli* BST Library (ver. 1-20) and shown in Table 2. The 33 isolates were comprised of individual fecal samples from cattle (1), goat (1), domestic cat (1), sewage (4), septic (6), feral hogs, armadillo, opossum, raccoon, squirrel, and deer (19), and seagull (1). The updated library contains 1,886 *E. coli* isolates from 1,645 different fecal samples representing over 50 animal subclasses. This is the result of collecting over 3,500 domestic sewage, wildlife, livestock, and pet fecal samples from 13 watersheds across Texas and screening over 6,000 isolates for clones and host specificity.

Source Categories

The updated Texas *E. coli* BST Library (ver. 1-20) was used to identify the source classes for water isolates in the watershed. Although fingerprint profiles were considered a match to a single entry, identification was to the host source class, and not to the individual animal represented by the best match. With currently available BST methods, it is not possible to discriminate between all possible species of animal sources simultaneously, since each added category tends to decrease the accuracy of source classification. This is illustrated in Table 1 where the 7-way split has an overall lower average rate of correct classification (91%) than does the 3-way split (100%). Therefore, we currently combine most species into groups based upon similarity of their physiology and/or potential management. Below are the 3- and 7-way split categories that were used for categorizing *E. coli* isolates for this study and which we have most frequently used for characterizing watersheds:
3-way split

1. Domesticated animals and livestock (livestock and pets)
2. Wildlife (including feral hogs)
3. Humans

7-way split

1. Cattle
2. Other livestock, non-avian (non-avian livestock other than cattle; sheep, etc.)
3. Other livestock, avian (chickens, etc.)
4. Pets (dogs, cats)
5. Avian wildlife (ducks, geese, sparrows, etc.)
6. Non-avian wildlife (deer, feral hogs, coyotes, etc.)
7. Humans

For any *E. coli* isolate that could not be matched to a group in the Texas *E. coli* BST Library (using an 80% similarity cutoff), its source category was designated as being “unidentified.”

Table 1. Texas *E. coli* BST Library (ver. 1-20, cross-library validation) composition and rates of correct classification (RCCs) by Jackknife analysis of ERIC-RP composite data sets using an 80% similarity cutoff and 3- and 7-way splits.

Source Class	Number of Isolates	Number of Samples	Library Composition and Expected Random Rate of Correct Classification	Calculated Rate of Correct Classification (RCC)	RCC to Random Ratio***	Left Unidentified (unique patterns)
HUMAN	**426**	**361**	**23%**	**100**	**4.3**	**22%**
DOMESTIC ANIMALS	**548**	**503**	**29%**	**100**	**3.4**	**20%**
Pets	84	75	4%	84	21.0	42%
Cattle	245	226	13%	93	7.2	11%
Avian Livestock	96	84	5%	89	17.8	27%
Other Non-Avian Livestock	123	118	7%	90	12.9	15%
WILDLIFE	**912**	**781**	**48%**	**100**	**2.1**	**16%**
Avian Wildlife	273	251	14%	79	5.6	19%
Non-Avian Wildlife	639	530	34%	91	2.7	15%
%Overall	**1886**	**1645**		**ARCC** = **3-way 100%** **7-way 91%**		**19%**

*RARCC, expected random average rate of correct classification based on library composition

**ARCC = average rate of correct classification: the proportion of all identification attempts which were correctly identified to source class for the entire library, which is similar to the mean of the RCCs for all source classes when the number of isolates in each source class is similar

***An RCC/Random Ratio greater than 1.0 indicates that the rate of correct classification is better than random. For example, the rate of correct classification for human is 4.5-fold greater than random chance based on library composition.

Table 2. Known-Source Fecal Samples Collected and *E. coli* Isolated, Screened, Validated, Archived and Added to the Texas *E. coli* BST Library.

Source	Samples Collected	Samples (+) for *E.coli*	Isolates archived	Isolates screened by ERIC	Isolates RP in local library	Self-validated (isolate/sample)	TRW 1-20 (isolate/sample)
Human	**15**	**15**	**29**	**29**	**15**	**10/10**	**10/10**
Sewage	11	11	17	17	11	4/4	4/4
Septic	4	4	12	12	4	6/6	6/6
Cattle	**3**	**2**	**2**	**2**	**2**	**1/1**	**1/1**
Other non-avian livestock	**9**	**8**	**8**	**8**	**8**	**4/4**	**1/1**
Goat	4	3	3	3	3	3/3	1/1
Hog, Domestic	3	3	3	3	3	1/1	0/0
Horse	1	1	1	1	1	0/0	0/0
Sheep	1	1	1	1	1	0/0	0/0
Other avian livestock	**4**	**3**	**3**	**3**	**1**	**0/0**	**0/0**
Chicken	2	2	2	2	0	0/0	0/0
Peacock	1	1	1	1	1	0/0	0/0
Parrot	1	0	0	0	0	0/0	0/0
Pets	**13**	**10**	**16**	**16**	**9**	**7/7**	**1/1**
Cat	6	5	11	11	5	3/3	1/1
Dog	7	5	5	5	4	4/4	0/0
Avian Wildlife	**14**	**8**	**14**	**14**	**8**	**1/1**	**1/1**
Buzzard	1	1	1	1	1	0/0	0/0
Pelican	4	2	4	4	2	0/0	0/0
Seagull	9	5	9	9	5	1/1	1/1
Non-Avian Wildlife	**33**	**31**	**79**	**79**	**33**	**23/21**	**19/17**
Armadillo	1	1	3	3	2	2/1	2/1
Deer	7	6	16	16	6	5/5	4/4
Hog, Feral	13	13	37	37	14	9/8	7/6
Possum	7	6	16	16	6	4/4	4/4
Raccoon	3	3	5	5	3	2/2	1/1
Squirrel	2	2	2	2	2	1/1	1/1
Total	**91**	**77**	**151**	**151**	**76**	**46/44**	**33/31**

Results and Observations

In Tables 3-7, the far-right columns list the ID of the closest library match for each tested *E. coli* isolate and their percent similarity. The ID of the closest library match for each isolate should be used for informational purposes only and not be interpreted as species-level source classification of the isolates since our current methods are not capable of doing this (e.g., they cannot distinguish between isolates from different species of non-avian wildlife, such as deer and feral hogs).

Library-Dependent BST Results

Results from the sampling event collected between April 2018 and April 2019 from all five sites combined are shown in Figure 2.

Using a 3-way split, 56% of the isolates were classified as originating from wildlife, 16% from livestock and domesticated animals, and 8% from humans. Using the more detailed 7-way split, 36% of the isolates were classified as originating from non-avian wildlife, 20% from avian wildlife, 10% from cattle, 8% from humans, 2% for other non-avian livestock, 2% from pets, and 1% for other avian livestock. The source could not be identified for 20% of the isolates.

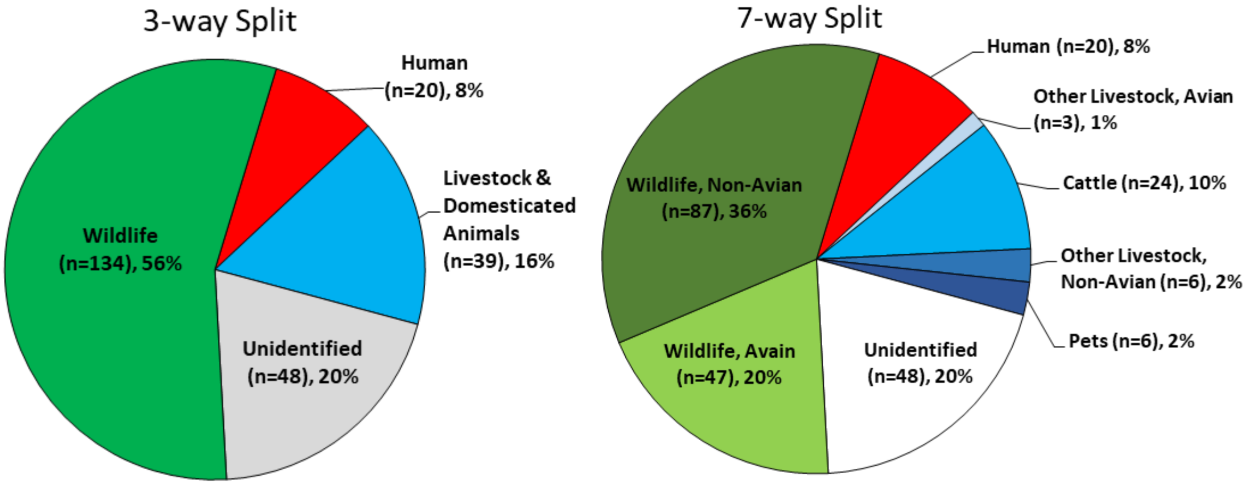

Figure 2. Source classification of *E. coli* isolates (combined n=241) from all five sampling sites using a 3-way split (L) and 7-way split (R).

Site TRW-11111 on Cedar Bayou

BST results for *E. coli* isolated from site TRW-11111 are shown in Figure 3 and Table 3. Using a 3-way split, isolates from this sample were classified as 58% originating from wildlife, 17% from humans, and 6% from livestock and domesticated animals. Using a 7-way split, the isolates were further characterized as 35% originating from non-avian wildlife and 23% from avian wildlife, 17% from humans, and 6% from cattle. The source could not be identified for 19% of the isolates. For each of the tested *E. coli* isolates, Table 3 lists the original source of their closest match in the Texas *E. coli* BST Library.

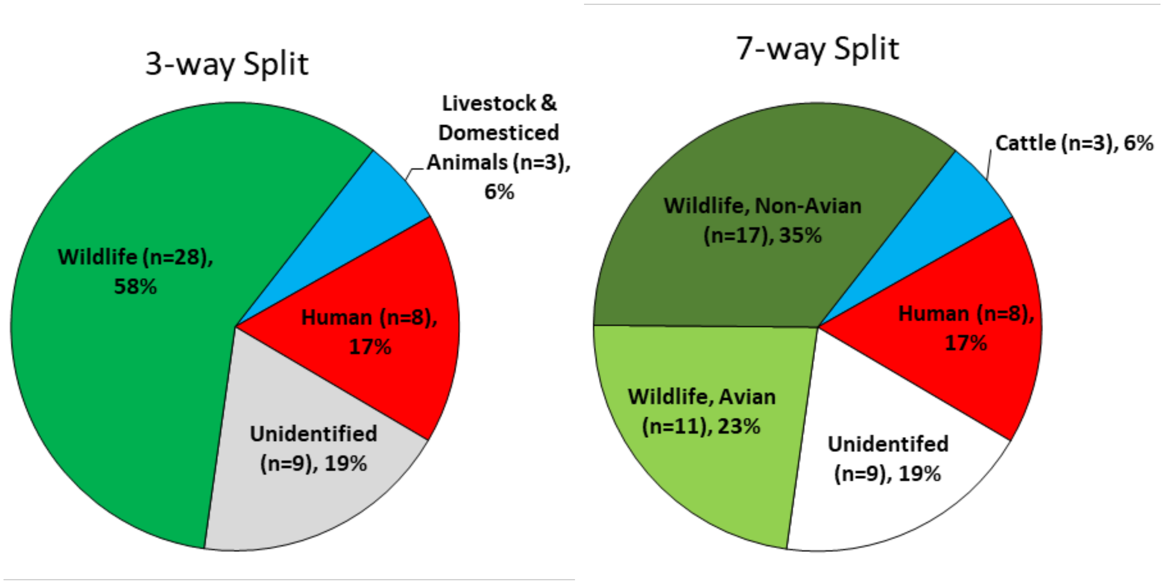

Figure 3. Source classification of *E. coli* isolates (combined n=48) from site TRW-11111 using a 3-way split (L) and 7-way split (R).

Table 3. Classification of *E. coli* isolates from site TRW-11111. *The ID of the closest library match for each isolate is provided for informational purposes only.

Isolate Key	3way id	7way id	Source of closest match*	Similarity (%)
TRW-0418-11111B	Wildlife	Wildlife, Avian	Cowbird	84.5
TRW-0418-11111C	Wildlife	Wildlife, Avian	Killdeer	92.9
TRW-0418-11111D	Wildlife	Wildlife, Non-Avian	Mouse	88.7
TRW-0418-11111E	Wildlife	Wildlife, Non-Avian	Coyote	91.5
TRW-0518-11111A	Human	Human	Human	81.7
TRW-0518-11111B	Human	Human	Human	86.5
TRW-0518-11111F	Wildlife	Wildlife, Non-Avian	Hog, Feral	89.0
TRW-0518-11111G	Human	Human	Human	86.1
TRW-0618-11111A	Wildlife	Wildlife, Non-Avian	Possum	82.0
TRW-0618-11111C	Wildlife	Wildlife, Avian	Pigeon	87.9
TRW-0618-11111D	Domesticated animals	Cattle	Cattle	93.6
TRW-0618-11111E	Wildlife	Wildlife, Non-Avian	Coyote	86.7
TRW-0718-11111D	Human	Human	Human	85.8
TRW-0718-11111E	Wildlife	Wildlife, Avian	Cliff Swallow/House Sparrow	88.3
TRW-0718-11111F	Wildlife	Wildlife, Non-Avian	Possum	86.9
TRW-0718-11111G	Wildlife	Wildlife, Non-Avian	Possum	93.4
TRW-0818-11111A	Unidentified	Unidentified	Human	78.3
TRW-0818-11111B	Unidentified	Unidentified	Human	77.8
TRW-0818-11111E	Unidentified	Unidentified	Human	76.1
TRW-0818-11111F	Unidentified	Unidentified	Human	76.4
TRW-1018-11111A	Wildlife	Wildlife, Avian	Cliff Swallow/House Sparrow	90.5
TRW-1018-11111D	Human	Human	Human	91.0
TRW-1018-11111E	Wildlife	Wildlife, Non-Avian	Raccoon	88.2
TRW-1018-11111F	Unidentified	Unidentified	Cattle	75.9
TRW-1118-11111B	Wildlife	Wildlife, Avian	Royal Tern	87.3
TRW-1118-11111C	Wildlife	Wildlife, Non-Avian	Possum	95.5
TRW-1118-11111D	Domesticated animals	Cattle	Cattle	88.6
TRW-1118-11111E	Wildlife	Wildlife, Non-Avian	Possum	88.0
TRW-1218-11111E	Wildlife	Wildlife, Non-Avian	Coyote	90.2
TRW-1218-11111F	Unidentified	Unidentified	Hog, Feral	77.6
TRW-1218-11111G	Unidentified	Unidentified	Hog, Feral	75.6
TRW-1218-11111H	Wildlife	Wildlife, Non-Avian	Hog, Feral	91.0
TRW-0119-11111B	Wildlife	Wildlife, Non-Avian	Possum	87.9
TRW-0119-11111C	Human	Human	Human	88.4
TRW-0119-11111D	Domesticated animals	Cattle	Cattle	88.9
TRW-0119-11111E	Wildlife	Wildlife, Non-Avian	Possum	84.5
TRW-0219-11111A	Wildlife	Wildlife, Non-Avian	Coyote	92.2
TRW-0219-11111B	Human	Human	Human	84.7
TRW-0219-11111E	Human	Human	Human	83.1
TRW-0219-11111F	Wildlife	Wildlife, Avian	Cowbird	91.9

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Table 3. Classification of *E. coli* isolates from site TRW-11111 (cont'd). *The ID of the closest library match for each isolate is provided for informational purposes only.

Isolate Key	3way id	7way id	Source of closest match*	Similarity (%)
TRW-0319-11111C	Wildlife	Wildlife, Non-Avian	Raccoon	89.6
TRW-0319-11111E	Wildlife	Wildlife, Avian	Turkey	89.6
TRW-0319-11111F	Wildlife	Wildlife, Avian	Heron	91.2
TRW-0319-11111G	Unidentified	Unidentified	Human	69.4
TRW-0419-11111A	Wildlife	Wildlife, Non-Avian	Possum	90.0
TRW-0419-11111G	Wildlife	Wildlife, Avian	Cowbird	91.5
TRW-0419-11111H	Unidentified	Unidentified	Mouse	64.8
TRW-0419-11111O	Wildlife	Wildlife, Avian	Egret	85.0

Site TRW-11271 on Buffalo Bayou

BST results for *E. coli* isolated from site TRW-11271 are shown in Figure 4 and Table 4. Using a 3-way split, isolates from this sample were classified as 55% originating from wildlife, 22% from livestock and domesticated animals, and 10% from humans. Using a 7-way split, the isolates were further characterized as 37% originating from non-avian wildlife and 18% from avian wildlife, 12% from cattle, 10% from humans, 4% other avian livestock, 4% from pets, and 2% other non-avian livestock. The source could not be identified for 12% of the isolates. For each of the tested *E. coli* isolates, Table 4 lists the original source of their closest match in the Texas *E. coli* BST Library.

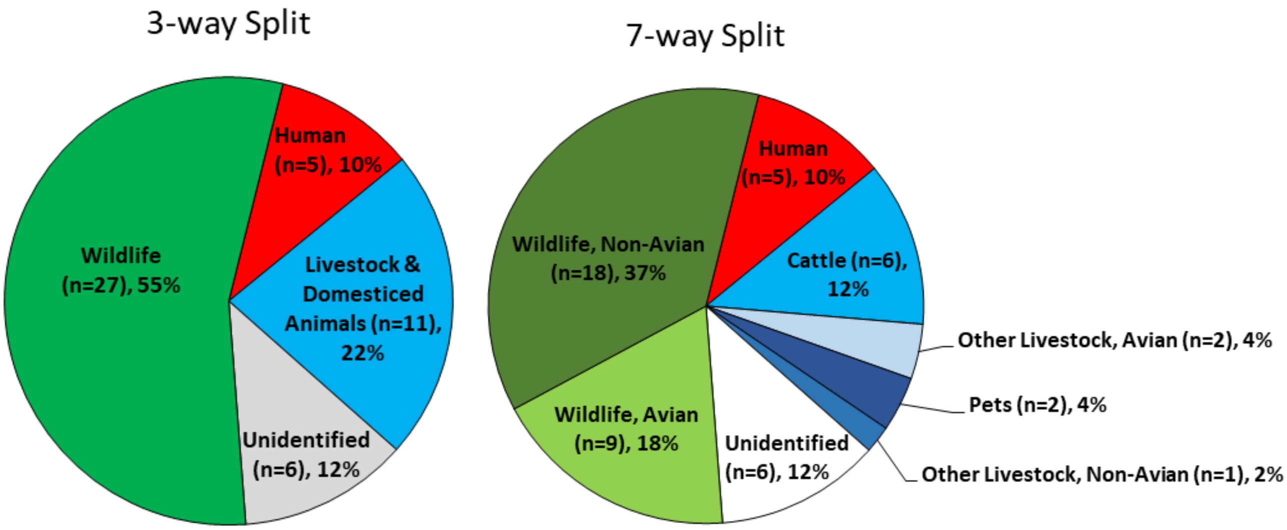

Figure 4. Source classification of *E. coli* isolates (combined n=49) from site TRW-11271 using a 3-way split (L) and 7-way split (R).

Table 4. Classification of *E. coli* isolates from site TRW-11271. *The ID of the closest library match for each isolate is provided for informational purposes only.

Isolate Key	3way id	7way id	Source of closest match*	Similarity (%)
TRW-0418-11271A	Wildlife	Wildlife, non-avian	Hog, Feral	90.1
TRW-0418-11271C	Human	Human	Human	91.2
TRW-0418-11271D	Wildlife	Wildlife, avain	Duck	86.9
TRW-0418-11271E	Wildlife	Wildlife, avain	Cliff Swallow	82.1
TRW-0518-11271A	Wildlife	Wildlife, non-avian	Opossum	91.2
TRW-0518-11271B	Domesticated animals	Cattle	Cattle	90.1
TRW-0518-11271C	Wildlife	Wildlife, non-avian	Opossum	93.2
TRW-0518-11271E	Domesticated animals	Cattle	Cattle	95.0
TRW-0518-11271F	Domesticated animals	Cattle	Cattle	89.6
TRW-0618-11271A	Wildlife	Wildlife, avain	Cliff Swallow/House Sparrow	92.2
TRW-0618-11271B	Unidentified	Unidentified	Deer	78.7
TRW-0618-11271D	Wildlife	Wildlife, non-avian	Raccoon	90.4
TRW-0618-11271E	Domesticated animals	Pets	Dog	86.7
TRW-0718-11271C	Domesticated animals	Other livestock, avian	Chicken	82.4
TRW-0718-11271D	Wildlife	Wildlife, non-avian	Mouse	92.9
TRW-0718-11271E	Wildlife	Wildlife, non-avian	Raccoon	94.2
TRW-0718-11271F	Domesticated animals	Cattle	Cattle	90.9
TRW-0818-11271A	Human	Human	Human	90.1
TRW-0818-11271B	Wildlife	Wildlife, non-avian	Opossum	88.3
TRW-0818-11271C	Domesticated animals	Cattle	Cattle	93.7
TRW-0818-11271D	Wildlife	Wildlife, avain	Cliff Swallow/House Sparrow	91.0
TRW-1018-11271A	Unidentified	Unidentified	Human	67.4
TRW-1018-11271B	Wildlife	Wildlife, non-avian	Coyote	91.3
TRW-1018-11271C	Wildlife	Wildlife, non-avian	Mouse	89.8
TRW-1018-11271D	Wildlife	Wildlife, non-avian	Deer	89.8
TRW-1018-11271E	Wildlife	Wildlife, non-avian	Javelina	91.5
TRW-1018-11271G	Domesticated animals	Other livestock, non-avian	Hog, Domestic	88.9
TRW-1118-11271A	Wildlife	Wildlife, avain	Cowbird	94.5
TRW-1118-11271D	Unidentified	Unidentified	Duck	75.2
TRW-1118-11271E	Human	Human	Human	88.9
TRW-1118-11271F	Wildlife	Wildlife, non-avian	Raccoon	92.3
TRW-1218-11271C	Wildlife	Wildlife, non-avian	Coyote	89.6
TRW-1218-11271E	Domesticated animals	Pets	Dog	85.0
TRW-1218-11271F	Wildlife	Wildlife, non-avian	Hog, Feral	87.2
TRW-1218-11271i	Unidentified	Unidentified	Hog, Feral	75.2
TRW-0119-11271A	Unidentified	Unidentified	Duck	74.7
TRW-0119-11271C	Wildlife	Wildlife, non-avian	Hog, Feral	87.3
TRW-0119-11271G	Wildlife	Wildlife, non-avian	Hog, Feral	86.7
TRW-0119-11271i	Unidentified	Unidentified	Human	72.9
TRW-0219-11271A	Domesticated animals	Cattle	Cattle	83.3
TRW-0219-11271C	Wildlife	Wildlife, avain	Duck	92.6
TRW-0219-11271D	Wildlife	Wildlife, avain	Cowbird	94.0
TRW-0219-11271G	Human	Human	Human	92.6

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Table 4. Classification of *E. coli* isolates from site TRW-11271 (cont'd). *The ID of the closest library match for each isolate is provided for informational purposes only.

Isolate Key	3way id	7way id	Source of closest match*	Similarity (%)
TRW-0319-11271E	Domesticated animals	Other livestock, avian	Chicken	89.0
TRW-0319-11271G	Wildlife	Wildlife, non-avian	Opossum	86.0
TRW-0419-11271A	Wildlife	Wildlife, avain	Heron	92.8
TRW-0419-11271C	Wildlife	Wildlife, non-avian	Opossum	91.2
TRW-0419-11271F	Wildlife	Wildlife, avain	Heron	95.2
TRW-0419-11271G	Human	Human	Human	80.5

Site TRW-11460 on Dickinson Bayou

BST results for *E. coli* isolated from site TRW-11460 are shown in Figure 5 and Table 5. Using a 3-way split, isolates from this sample were classified as 65% originating from wildlife, 17% from livestock and domesticated animals, and 4% from humans. Using a 7-way split, the isolates were further characterized as 48% originating from non-avian wildlife and 17% from avian wildlife, 10% from cattle, 4% from humans, 2% other avian livestock, 2% other non-avian livestock, and 2% from pets. The source could not be identified for 15% of the isolates. For each of the tested *E. coli* isolates, Table 5 lists the original source of their closest match in the Texas *E. coli* BST Library.

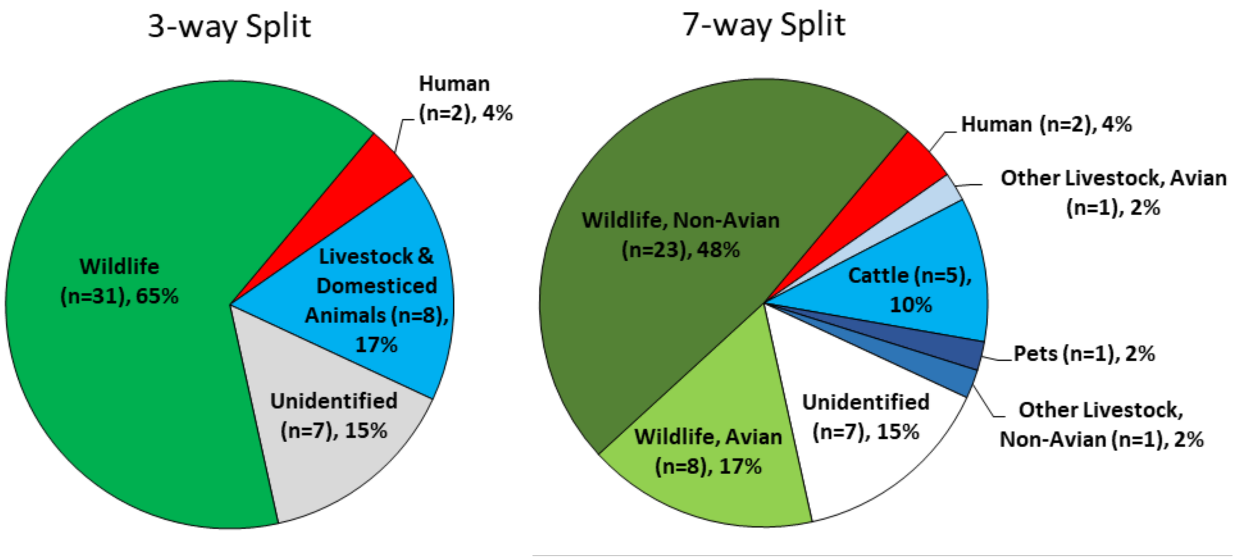

Figure 5. Source classification of *E. coli* isolates (combined n=48) from site TRW-11460 using a 3-way split (L) and 7-way split (R).

Table 5. Classification of *E. coli* isolates from site TRW-11460. *The ID of the closest library match for each isolate is provided for informational purposes only.

Isolate Key	3way id	7way id	Source of closest match*	Similarity (%)
TRW-0418-11460A	Wildlife	Wildlife, non-avian	Hog, Feral	80.1
TRW-0518-11460A	Human	Human	Human	82.7
TRW-0518-11460B	Wildlife	Wildlife, avain	Duck	80.5
TRW-0518-11460C	Unidentified	Unidentified	Duck	73.8
TRW-0518-11460D	Domesticated animals	Cattle	Duck	85.1
TRW-0618-11460C	Wildlife	Wildlife, non-avian	Coyote	89.2
TRW-0618-11460E	Wildlife	Wildlife, avain	Cliff Swallow	82.1
TRW-0618-11460F	Domesticated animals	Cattle	Cattle	93.8
TRW-0618-11460G	Wildlife	Wildlife, non-avian	Deer	84.0
TRW-0718-11460A	Wildlife	Wildlife, non-avian	Coyote	91.6
TRW-0718-11460B	Unidentified	Unidentified	Dog	67.6
TRW-0718-11460C	Wildlife	Wildlife, avain	Cliff Swallow/House Sparrow	87.8
TRW-0718-11460G	Wildlife	Wildlife, non-avian	Raccoon	86.9
TRW-0818-11460A	Wildlife	Wildlife, non-avian	Opossum	88.3
TRW-0818-11460B	Wildlife	Wildlife, non-avian	Hog, Feral	89.3
TRW-0818-11460C	Wildlife	Wildlife, avain	Turkey	88.0
TRW-0818-11460F	Unidentified	Unidentified	Sheep	70.5
TRW-1018-11460B	Domesticated animals	Pets	Cat	92.3
TRW-1018-11460D	Wildlife	Wildlife, non-avian	Coyote/Fox	91.8
TRW-1018-11460E	Wildlife	Wildlife, avain	Cliff Swallow/House Sparrow	86.7
TRW-1018-11460F	Wildlife	Wildlife, avain	Cliff Swallow/House Sparrow	88.3
TRW-1118-11460A	Wildlife	Wildlife, non-avian	Hog, Feral	81.9
TRW-1118-11460B	Domesticated animals	Cattle	Cattle	85.1
TRW-1118-11460D	Domesticated animals	Cattle	Cattle	89.3
TRW-1118-11460E	Wildlife	Wildlife, non-avian	Squirrel	91.9
TRW-1218-11460A	Unidentified	Unidentified	Cattle	78.9
TRW-1218-11460C	Unidentified	Unidentified	Cattle	74.5
TRW-1218-11460D	Wildlife	Wildlife, non-avian	Deer	92.2
TRW-1218-11460E	Wildlife	Wildlife, non-avian	Hog, Feral	85.1
TRW-0119-11460A	Wildlife	Wildlife, non-avian	Opossum	84.8
TRW-0119-11460B	Wildlife	Wildlife, avain	Pigeon	90.3
TRW-0119-11460C	Wildlife	Wildlife, non-avian	Armadillo	92.6
TRW-0119-11460D	Wildlife	Wildlife, avain	Pigeon	91.2
TRW-0119-11460E	Wildlife	Wildlife, non-avian	Mouse	90.3
TRW-0219-11460A	Wildlife	Wildlife, non-avian	Hog, Feral	92.4
TRW-0219-11460C	Unidentified	Unidentified	Human	78.9
TRW-0219-11460E	Human	Human	Human	91.6
TRW-0219-11460F	Wildlife	Wildlife, non-avian	Mouse	85.4
TRW-0219-11460G	Wildlife	Wildlife, non-avian	Mouse	88.4

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Table 5. Classification of *E. coli* isolates from site TRW-11460 (cont'd). *The ID of the closest library match for each isolate is provided for informational purposes only.

Isolate Key	3way id	7way id	Source of closest match*	Similarity (%)
TRW-0319-11460C	Domesticated animals	Cattle	Cattle	84.8
TRW-0319-11460F	Unidentified	Unidentified	Prairie Dog	72.3
TRW-0319-11460H	Wildlife	Wildlife, non-avian	Coyote	86.7
TRW-0319-11460i	Domesticated animals	Other livestock, avian	Chicken	89.1
TRW-0419-11460A	Wildlife	Wildlife, non-avian	Coyote	90.9
TRW-0419-11460B	Domesticated animals	Other livestock, non-avian	Hog, Domestic	87.1
TRW-0419-11460C	Wildlife	Wildlife, non-avian	Raccoon	96.9
TRW-0419-11460D	Wildlife	Wildlife, non-avian	Coyote/Fox	93.3
TRW-0419-11460H	Wildlife	Wildlife, non-avian	Hog, Feral	88.9

Site TRW-14552 on Double Bayou

BST results for *E. coli* isolated from site TRW-14552 are shown in Figure 6 and Table 6. Using a 3-way split, isolates from this sample were classified as 52% originating from wildlife, 19% from livestock and domesticated animals, and 4% from humans. Using a 7-way split, the isolates were further characterized as 31% originating from non-avian wildlife, 21% from avian wildlife, 17% from cattle, 4% from humans, and 2% from pets. The source could not be identified for 25% of the isolates. For each of the tested *E. coli* isolates, Table 6 lists the original source of their closest match in the Texas *E. coli* BST Library.

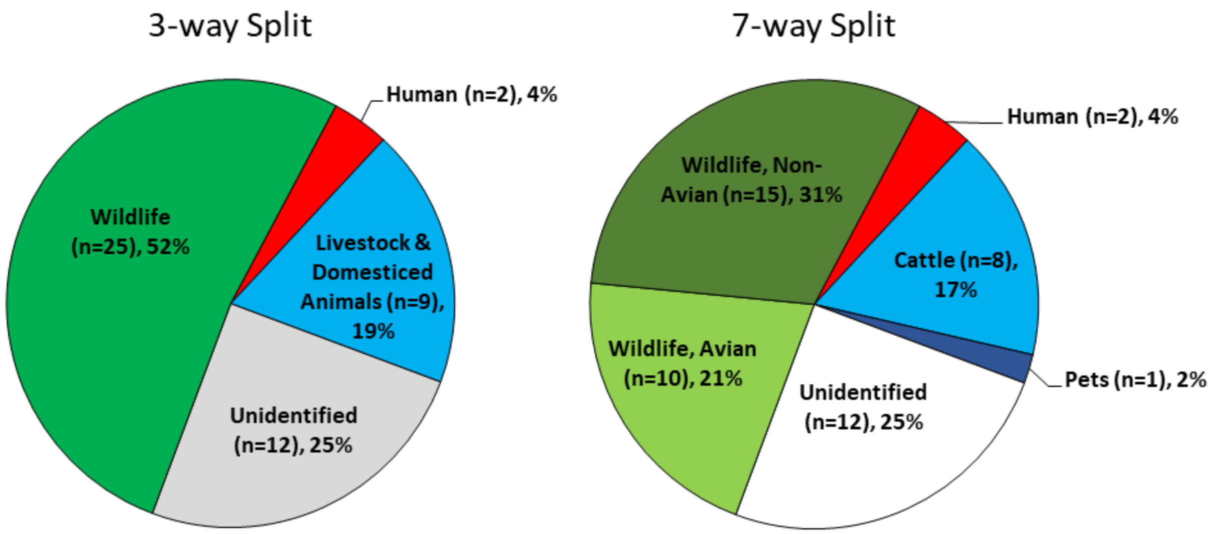

Figure 6. Source classification of *E. coli* isolates (combined n=48) from site TRW-14552 using a 3-way split (L) and 7-way split (R).

Table 6. Classification of *E. coli* isolates from site TRW-14552. *The ID of the closest library match for each isolate is provided for informational purposes only.

Isolate Key	3way id	7way id	Source of closest match*	Similarity (%)
TRW-0418-14552A	Wildlife	Wildlife, non-avian	Hog, Feral	88.1
TRW-0418-14552B	Domesticated animals	Cattle	Cattle	80.7
TRW-0418-14552C	Domesticated animals	Cattle	Cattle	91.2
TRW-0418-14552D	Unidentified	Unidentified	Coyote	69.9
TRW-0518-14552B	Domesticated animals	Cattle	Cattle	81.5
TRW-0518-14552C	Unidentified	Unidentified	Chicken	72.9
TRW-0518-14552D	Unidentified	Unidentified	Pigeon	79.2
TRW-0518-14552F	Unidentified	Unidentified	Goat	78.3
TRW-0618-14552A	Wildlife	Wildlife, avain	Cliff Swallow/House Sparrow	91.2
TRW-0618-14552C	Wildlife	Wildlife, avain	Heron	94.4
TRW-0618-14552D	Domesticated animals	Pets	Dog	83.4
TRW-0618-14552E	Wildlife	Wildlife, avain	Turkey	89.6
TRW-0718-14552C	Wildlife	Wildlife, non-avian	Javelina	84.2
TRW-0718-14552D	Wildlife	Wildlife, non-avian	Javelina	86.2
TRW-0718-14552E	Wildlife	Wildlife, non-avian	Coyote	89.6
TRW-0718-14552F	Human	Human	Human	91.1
TRW-0818-14552B	Wildlife	Wildlife, non-avian	Raccoon	86.2
TRW-0818-14552C	Wildlife	Wildlife, non-avian	Hog, Feral	88.6
TRW-0818-14552F	Unidentified	Unidentified	Dog	79.8
TRW-0818-14552G	Wildlife	Wildlife, non-avian	Coyote	81.9
TRW-1018-14552A	Human	Human	Human	83.4
TRW-1018-14552B	Domesticated animals	Cattle	Cattle	90.1
TRW-1018-14552D	Wildlife	Wildlife, non-avian	Coyote	89.8
TRW-1018-14552F	Unidentified	Unidentified	Human	72.9
TRW-1118-14552C	Domesticated animals	Cattle	Cattle	91.4
TRW-1118-14552D	Unidentified	Unidentified	Mouse	77.5
TRW-1118-14552E	Wildlife	Wildlife, avain	Goose	93.9
TRW-1118-14552G	Wildlife	Wildlife, non-avian	Opossum	84.1
TRW-1218-14552D	Unidentified	Unidentified	Buzzard	79.8
TRW-1218-14552F	Wildlife	Wildlife, avain	Goose	86.3
TRW-1218-14552H	Wildlife	Wildlife, non-avian	Raccoon	85.6
TRW-1218-14552i	Wildlife	Wildlife, non-avian	Opossum	87.5
TRW-0119-14552A	Wildlife	Wildlife, non-avian	Rat	88.0
TRW-0119-14552C	Wildlife	Wildlife, avain	Cliff Swallow	81.9
TRW-0119-14552D	Wildlife	Wildlife, non-avian	Hog, Feral	89.7
TRW-0119-14552E	Wildlife	Wildlife, non-avian	Opossum	87.6
TRW-0219-14552A	Unidentified	Unidentified	Pigeon	76.9
TRW-0219-14552B	Domesticated animals	Cattle	Cattle	88.5
TRW-0219-14552C	Unidentified	Unidentified	Raccoon	66.9
TRW-0219-14552G	Wildlife	Wildlife, non-avian	Coyote	80.8

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Table 6. Classification of *E. coli* isolates from site TRW-14552 (cont'd). *The ID of the closest library match for each isolate is provided for informational purposes only.

Isolate Key	3way id	7way id	Source of closest match*	Similarity (%)
TRW-0319-14552A	Domesticated animals	Cattle	Cattle	89.2
TRW-0319-14552F	Wildlife	Wildlife, avain	Turkey	88.9
TRW-0319-14552G	Wildlife	Wildlife, avain	Turkey	91.3
TRW-0319-14552H	Wildlife	Wildlife, avain	Turkey	91.0
TRW-0419-14552F	Wildlife	Wildlife, avain	Cliff Swallow/House Sparrow	91.4
TRW-0419-14552L	Domesticated animals	Cattle	Cattle	88.8
TRW-0419-14552M	Unidentified	Unidentified	Donkey	61.6
TRW-0419-14552N	Unidentified	Unidentified	Coyote	79.7

Site TRW-16575 on Clear Creek

BST results for *E. coli* isolated from site TRW-16575 are shown in Figure 7 and Table 7. Using a 3-way split, isolates from this sample were classified as 47% originating from wildlife, 16% from livestock and domesticated animals, and 6% from humans. Using a 7-way split, the isolates were further characterized as 29% originating from non-avian wildlife, 18% from avian wildlife, 8% other non-avian livestock, 6% from humans, 4% from cattle, and 4% from pets. The source could not be identified for 31% of the isolates. For each of the tested *E. coli* isolates, Table 7 lists the original source of their closest match in the Texas *E. coli* BST Library.

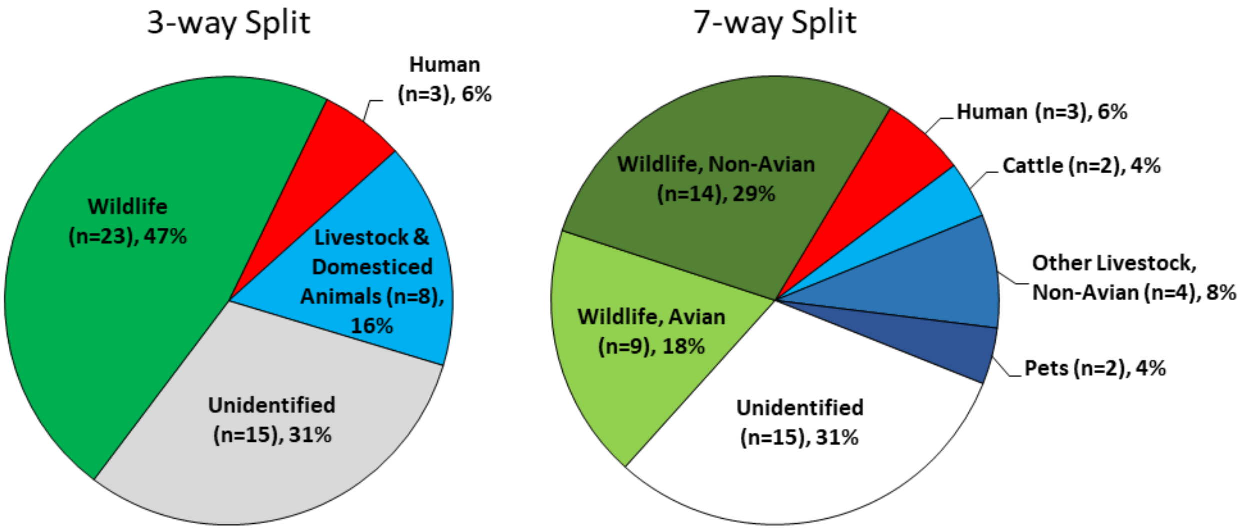

Figure 7. Source classification of *E. coli* isolates (combined n=48) from site TRW-16575 using a 3-way split (L) and 7-way split (R).

Table 7. Classification of *E. coli* isolates from site TRW-16575. *The ID of the closest library match for each isolate is provided for informational purposes only.

Isolate Key	3way id	7way id	Source of closest match*	Similarity (%)
TRW-0418-16575A	Human	Human	Human	81.2
TRW-0418-16575B	Wildlife	Wildlife, non-avian	Skunk	89.8
TRW-0418-16575E	Wildlife	Wildlife, non-avian	Porcupine	84.8
TRW-0418-16575F	Unidentified	Unidentified	Fox	74.9
TRW-0518-16575A	Unidentified	Unidentified	Coyote	73.3
TRW-0518-16575B	Unidentified	Unidentified	Cattle	67.3
TRW-0518-16575C	Unidentified	Unidentified	Sheep	70.2
TRW-0518-16575D	Unidentified	Unidentified	Deer	76.0
TRW-0618-16575C	Unidentified	Unidentified	Hog, Feral	79.6
TRW-0618-16575D	Wildlife	Wildlife, avain	Pigeon	92.0
TRW-0618-16575E	Unidentified	Unidentified	Cattle	79.9
TRW-0618-16575F	Domesticated animals	Other livestock, non-avian	Sheep	91.7
TRW-0718-16575B	Human	Human	Human	85.2
TRW-0718-16575C	Wildlife	Wildlife, non-avian	Opossum	92.0
TRW-0718-16575D	Wildlife	Wildlife, avain	Turkey	89.6
TRW-0718-16575G	Wildlife	Wildlife, non-avian	Deer	95.2
TRW-0818-16575A	Wildlife	Wildlife, non-avian	Mouse	89.6
TRW-0818-16575E	Wildlife	Wildlife, non-avian	Deer	88.2
TRW-0818-16575F	Unidentified	Unidentified	Human	72.7
TRW-0818-16575G	Unidentified	Unidentified	Sheep	74.9
TRW-1018-16575A	Wildlife	Wildlife, non-avian	Jack Rabbit	90.6
TRW-1018-16575C	Wildlife	Wildlife, non-avian	Opossum	92.4
TRW-1018-16575D	Wildlife	Wildlife, avain	Duck	93.5
TRW-1018-16575G	Unidentified	Unidentified	Hog, Feral	77.9
TRW-1118-16575A	Wildlife	Wildlife, non-avian	Fox	81.9
TRW-1118-16575B	Unidentified	Unidentified	Fox	78.9
TRW-1118-16575D	Wildlife	Wildlife, avain	Pelican	94.1
TRW-1118-16575E	Domesticated animals	Cattle	Cattle	86.5
TRW-1218-16575B	Unidentified	Unidentified	Hog, Feral	63.9
TRW-1218-16575D	Unidentified	Unidentified	Raccoon or Human	74.8
TRW-1218-16575E	Unidentified	Unidentified	Opossum	76.9
TRW-1218-16575H	Domesticated animals	Other livestock, non-avian	Sheep	92.3
TRW-0119-16575D	Domesticated animals	Other livestock, non-avian	Horse	92.6
TRW-0119-16575F	Wildlife	Wildlife, non-avian	Opossum	87.7
TRW-0119-16575G	Wildlife	Wildlife, avain	Pigeon	87.2
TRW-0119-16575H	Domesticated animals	Pets	Dog	83.8
TRW-0219-16575A	Wildlife	Wildlife, non-avian	Raccoon	91.5
TRW-0219-16575B	Wildlife	Wildlife, non-avian	Mouse	87.0
TRW-0219-16575C	Unidentified	Unidentified	Raccoon	76.3
TRW-0219-16575G	Wildlife	Wildlife, avain	Turkey	91.1

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Table 7. Classification of *E. coli* isolates from site TRW-16575 (cont'd). *The ID of the closest library match for each isolate is provided for informational purposes only.

Isolate Key	3way id	7way id	Source of closest match*	Similarity (%)
TRW-0319-16575A	Wildlife	Wildlife, avain	Turkey	92.2
TRW-0319-16575B	Domesticated animals	Pets	Dog	85.9
TRW-0319-16575C	Wildlife	Wildlife, avain	Duck	93.0
TRW-0319-16575i	Domesticated animals	Cattle	Cattle	88.1
TRW-0419-16575A	Wildlife	Wildlife, non-avian	Opossum	85.7
TRW-0419-16575D	Wildlife	Wildlife, non-avian	Coyote	91.6
TRW-0419-16575E	Wildlife	Wildlife, avain	Duck	92.9
TRW-0419-16575G	Human	Human	Human	87.1

Summary

Library-dependent BST was utilized to characterize sources of fecal contamination in tributaries of the Trinity and Galveston Bays. Water samples from five sites were collected between April 2018 and April 2019. The *E. coli* results from 241 isolates indicated a combination of wildlife and domesticated animals are the major source contributors of bacterial contamination in the watershed with variable contribution from human sources. Overall, the majority of the *E. coli* isolates (56%) were classified as originating from wildlife. This included substantial contributions from both non-avian wildlife sources, such as feral hogs, raccoons, opossums, mice and other small mammals, along with a variety of avian wildlife sources. Cattle, other livestock such as chickens, and pets made up another 15% of the total isolates identified. Human sources of *E. coli* made up 8% of the total classifications.

Relative source contributions were relatively consistent for sites 11271, 11460, 14452, and 16575 with 48-65% of *E. coli* being attributed to wildlife and 15-20% to livestock and domestic animal. Human contributions were more variable ranging from 4% of isolates at TRW-11460 and TRW-14552 to 17% of isolates at TRW-11111.

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